1 585 863

PATENT SPECIFICATION

(11) 1 585 863

(21) Application No. 35599/77 (22) Filed 24 Aug. 1977
(44) Complete Specification Published 11 Mar. 1981
(51) INT. CL³ A61K 35/74
(52) Index at Acceptance
A5B 170 180 190 21X 21Y 30X 30Y 312 31Y 327 381 38Y H
C6F 101 GC

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(54) PHARMACEUTICAL LACTOBACILLUS PREPARATIONS

(71) We, SEIKEN KAI FOUNDATIONAL JURIDICAL PERSON a legal body organized under the laws of Japan of No. 3-44, Matsuzaki-cho, 2-chome, Abeno-ku Osaka-shi. Osaka, Japan do hereby declare this invention for which we pray that a Patent may be granted to us, and the method by which it is to be performed to be particularly described in and by the following statement:-

This invention relates to a pharmaceutical preparation which is primarily useful for the prevention of infection or inflammation or combatting of inflammation or infectious diseases, i.e., one of the types of diseases which are hard to cure even by modern medical scientific methods.

According to one aspect of the present invention, there is provided a pharmaceutical *Lactobacillus* preparation useful for the prevention of infection or inflammation or combatting of inflammation or infectious disease comprising one or more strains of live *Lactobacillus* whose growth is enabled or promoted by addition of one or more of sodium sulphide, ammonia and acetic acid to at least one of Stephenson-Whetham medium, Stephenson-Whetham medium containing vitamins and Stephenson-Whetham medium containing casamino acid, said preparation being substantially free of other bacterial strains.

According to another aspect of the present invention, there is provided a pharmaceutical *Lactobacillus* preparation useful for the prevention of infection or inflammation or combatting of inflammation or infectious disease comprising one or more strains of live *Lactobacillus* whose growth is enabled or promoted by addition of one or more of sodium sulphide, ammonia and acetic acid to at least one of Stephenson-Whetham medium, Stephenson-Whetham medium containing vitamins and Stephenson-Whetham medium containing casamino acid; and a carrier and/or excipient.

According to a further aspect of the present invention, there is provided a method of treatment of a non-human mammal for the preparation of infection or inflammation or combatting of inflammation or infectious disease, comprising administering a preparation as defined in either of the last preceding two paragraphs to said non-human mammal.

Intrusion of bacteria into living bodies and their proliferation are referred to as "infection". Once bacteria start proliferating in the living bodies or the latter start showing reactions (called "infectious diseases") thereto, various symptons may be observed such as fever, flare and swelling. The conditions of the infectious diseases may take a turn for the better when medicines such as antibiotics are used adequately at this state. However, since administration of the antibiotic too late or in inadequate dosages, premature discontinuance of antibiotic treatment, the consumption of alcoholic beverages or other unexpected conduct by the patient, and various other phenomena (e.g. the phenomenon that the antibiotic does not adequately reach the effected part) can prevent the extermination or removal of bacteria from the infected living bodies, the medical treatment using antibiotics frequently proves unsuccessful. In some cases, the infectious disease may become chronic so that a cure is even more difficult by the present day's medical science. The *Lactobacillus* preparation of the present invention (hereinafter referred to as "the present preparation") is especially effective for the treatment of these diseases. By using typical conditions such as nasal inflammation; gastritis or enteritis, alveolar brennorrhea, pudendal laceration and hemorrhoids which are sometimes classified as incurable conditions, the use of the present preparation and examples of medical treatment using the

same are explained as follows.

(i) *Nasal Inflammation*

It is frequently observed that patients who catch cold and show nasal fluids discharge are, for some reasons, infected with pathogenic bacteria. Such bacteria proliferate profusely in the nasal sinus and subsequently start producing toxins. The bodily reaction to the toxins cause inflammation which, depending on its degree and type, generally induces the exudation of various substances. When the condition becomes serious, it may become extremely difficult to treat because the nasal fluids become more and more mucous and antibiotics have difficulty in penetrating such mucus or pus. Moreover, even the use of antibiotics in combination with anti-inflammatory enzymes is very often not effective.

Thus, in many cases, nasal inflammation cannot be cured by the use of antibiotics alone. Further, antibiotic resistant bacteria may appear under such conditions and secondary reactions occur which result in the disease becoming more serious. Surgical excision is one of the medical treatments generally remaining under these circumstances. The adequate use of the present preparation (preferable in this case is such a preparation which is made of an antibiotic-producing strain) is effective irrespective to whether or not surgical treatment has been performed. Thus, when a large amount of the present preparation was applied to the inflammed part (repeatedly if necessary), bacterial substitution took place gradually and, depending on the condition of patients, the pathogenic bacteria appeared to lose their effect as early as the 2nd day or, at the latest, at about the 15th day. It was also observed that a gradual improvement occurred accompanying the abatement of disappearance of inflammation and swelling and the dissolution or decrease of pus and purification at the site of the disease. In this case, however, when compounds having bacteridical activity against Lactobacillus (e.g., horse radish, red pepper and curry) and medicines such as antibiotics intrude into nasal sinus, it sometimes happened that the condition of the disease took a turn for the worse. This is primarily due to a fact that these bactericidal compounds prevent the proliferation of Lactobacillus. In such cases, therefore, it is especially important to use the present preparation which has been previously rendered resistant to the above-mentioned compounds.

Nowadays, a combination of antibiotics and anti-inflammatory enzymes is used for the treatment of sinusitis etc. However, even this type of treatment may not be effective enough for patients exhibiting a very heavy pus discharge and surgical treatment may be subsequently performed. However, the present preparation can be used with effect even when the disease has taken a strong hold, without having to operate.

(ii) *Appendicitis*

Infection with pathogenic bacteria is one of the important causes of this disease. In appendicitis sufferers pathogenic bacteria which induce inflammation gradually leak out of the appendix into the upper- and lower-intestinal organs and usually remain there even after appendectomy. In such case, the remaining pathogenic bacteria near the site of the removed appendix survive inveterately. It sometimes happens that patients are infected with bacteria in hospitals. In a sense, such infections are unavoidable and, even though large amounts of antibiotics may be used, the proliferation of pathogenic bacteria cannot be sufficiently prevented. When the present preparation is employed in such situations, it can bring about an early recovery from the disease because the *Lactobacillus* strains can, as in the case of the inflammatory diseases, digest or denature the pathogenic bacteria remaining in the body after the operation and also cell or serum secretions or exudates produced by biophylaxis reactions. Anyway, as antibiotics may be frequently used under these situations, the *Lactobacillus* strains to be used should preferably be those which are resistant to such antibiotics.

(iii) *Gastritis and enteritis*

Even nowadays it is not rare for those patients (e.g. infants and the aged) having a poor resistance to such diseases, to die of marasmus, pathogenic bacteria-induced gastritis and enteritis as well as the inflammatory diseases caused by bacteria such as enteritis vibrio, dysentery bacteria or *Salmonella*. However, patients normally gradually improve in a few days by the adequate use of antibiotics. However, some of these pathogenic bacteria may be resistance to the antibiotics used. In the latter cases, the disease may not be improved by the use of antibiotics alone, but may become more chronic and frequently induce secondary diseases. Thus, it is of the greatest importance to cure the diseases before they become chronic. For this purpose, it is desirable to adminster to the patients a large amount of the present preparation, if required in combination with antibiotics. Moreover, even in the cases where the diseases may be improved in a few days by administration of a suitable drug, the use of the present preparation is recommended because it can bring about an

earlier recovery from the disease and at the same time sweep away all the causes thereof.

(iv) *Sputum:*

Sputum is, like pus, formed by the pathological reaction of living bodies. In addition, sputum itself stimulates the living bodies to form more sputum, so that the formation of sputum continues endlessly as the disease worsens. This phenomenon is fundamentally the same as in the case of sinusitis. Even in this case, the Lactobacillus strains of the present preparation can purify the sputum because, as observed in the intestine, they digest, decompose or denature the nutrients contained in the sputum.

(v) *Gingivitis:*

When the inflammed parts of the body are treated with, for example, antibiotics and anti-inflammatory enzymes, there is frequently little success and bacteria still remain alive without being dispelled by the antibiotics, anti-inflammatory enzymes and the biophylaxis reaction of the body. This is frequently the case with alveolar abscesses and, apart from the insufficient therapeutic effects of the drug employed, is due to the fact that the gum tissues are not restored completely at the time when there is a significant decrease in the number of cells of the pathogenic bacteria.

In such cases, the bacteria and pathogenic bacteria which remain in the oral cavity and in gaps in the teeth and gums, multiply to produce a relapse in the patient. Thus, in this type of disease, it is important to administer the present preparation when the amount of pathogenic bacteria has been decreased. By the administration of the present preparation, the quantitative ratio of the remaining pathogenic bacteria to the *Lactobacillus* strains of the present invention is reduced and simultaneously, the conditions or color of the gums which showed the inflammation swelling may be improved significantly.

In other words, when the parts infected must be treated urgently, antibiotics may be employed to kill pathogenic and non-pathogenic bacteria, and therafter the inflammation caused by the biophylaxis reaction is improved by the use of the present preparation.

(vi) *Hemorrhoids*

Many theories have been propounded in the past regarding the causes of this condition. However, the actual causes are now relatively widely accepted.

The main cause has a close relationship with infection by pathogenic bacteria, i.e., the kinds and quantity of the pathogenic bacteria; and another cause concerns the presence of intestinal bacteria, i.e. fecal infections; a third cause relates to the structure of the intestinal organs or the anus; a fourth cause relates to the degree and frequency of irritation of affected parts, i.e. constipation and solidity of excrement; a fifth cause relates to the mode of the bodily reaction (i.e., the conditions of the wound) and the recuperative ability (i.e. physical constitution); and a sixth cause relates to the degree and scope of blood cogestion. The wound or laceration and bacterial infection are peculiar to hemerrhoids and it is rare for patients to be afflicted, on account of laceration alone, with serious hemorrhoids which affects their daily life. Thus, hemorrhoids can be considered to be the inflammation of the anus and its surrounding portions caused primarily by bacterial infection or by combination thereof with various other of the above causes. Further, the condition and degree of the condition may vary depending on the bacteria, the interrelation with intestinal bacteria, the reactivity of the body, the degree of irritation and the site of the affected part. However, it is an undeniable fact that, in almost all cases of hemorrhoids, bacterial infection plays an important role. In this sense, therefore, it should be recognized that hemorrhoids is indicative of bacterial infection. Based on this understanding, hemorrhoids should be considered, like laryngitis or various inflammation caused by laceration of the birth channel, as the most complicated and unclassable type of inflammation which is primarily caused by bacterial infection.

Hemorrhoids is one of the most incurable diseases and the difficulty in ascertaining the types of pathogenic bacteria which are present, the resistance thereof to drugs, the degree of local osmosis of the pathogenic bacteria and infectiousness thereof render even the choice of drugs quite difficult.

Further, whether a drug is in fact effective for the intestinal bacteria in the body must be decided independently of the bactericidal activity of the drug because the body is: in any event, infected with huge amounts of various intestinal bacteria. These two points make the chose of suitable drugs more difficult.

To sum up, first of all, the *Lactobacillus* strains which are used in the present preparation have a characteristic ability to control or prevent the growth of other living microorganisms. This fact has been proven during investigations relating to deodorizing excrement. This ability also serves to prevent the growth of, to kill, or to induce bacterial substitution of, the pathogenic bacteria which form the focus of disease in the afflicted part. Moreover, said

ability also works effectively against intestinal bacteria. Further, since the *Lactobacillus* strains of the present preparation kill the bacteria found on and around the inflammed portion and have a protective effect against intestinal bacteria, and also since the balance of power between the *Lactobacillus* and the intestinal bacteria is shifted in favor of the former thereby decreasing the amount of putretative bacteria in the intestine, the above-mentioned ability of the *Lactobacillus* strains can lighten the burden which the living bodies must bear for the purpose of phylaxis. In addition, the strong purifying ability of the present preparation has already been proven through the experiments concerning the deodorization of odorous materials.

Further, the strains used in preparing the present preparation include those having strong antibiotic-productivity. In such cases, the present preparation exhibits a very potent ability to prevent the growth of pathogenic or non-pathogenic bacteria and, as a result, hemorrhoids can be frequently improved, or if not serious, cured almost completely by the use of the present preparation.

Hemorrhoids is characterised by a severe irritation and a contamination of the affected parts. Thus, diseases which affect the host so adversely as hemorrhoids are relatively rare. Such diseases are usually found in infected eyes, the oral cavity, the gums, the throat, the abdominal cavity or the sexual organs, or, in the case of operation patients, in the field of gynaecology.

Concomitantly, although in the foregoing description, pathogenic bacteria have been described as being mainly responsible for hemorrhoids, it is to be noted that hemorrhoids can sometimes be induced by intestinal pathogenic bacteria.

(vii) *Pudendal laceration at the time of childbirth*

Oral administration of the present preparation or the direct application thereof to the affected part is useful for improving the inflammatory symptoms including swelling, flare or pain in the affected area.

We carried out experiments using various Lactobacillus strains, some of which are resistant to drugs or popular condiments and others not. In order to check whether the present preparation had exerted its effects sufficiently, it had first to be ascertained that the inflammation of the living bodies had disappeared completely. However, since chronic inflammatory diseases cannot be cured in a short period, it is virtually impossible with the present day's food to continue living without taking any condiments until the complete recovery from the disease. For this reason, therefore, in making the present preparation, it is important to use *Lactobacillus* strains which are resistant to condiments or antibiotics.

The extensive experiments were carried out although only some of these experiments are described herein.

Since the development of bactericidal agents and antibiotics including red prontosil and penicillins, these products have been used extensively for the treatment of bacteria-induced diseases because of their effectiveness compared with previous treatments. This is primarily because, when pyogenic infection or suppurative inflammation is present, the *Lactobacillus* strains of the present preparation can assimilate or denature and finally purify various exudates which are formed at the site where inflammation is observed due to combat between the host organism and various foreign bodies. The present preparation has a high effectiveness and a wide applicability which are almost comparable to those of sulfa agents, antibiotics and anti-inflammatory enzymes. This is primarily because in the case of suppurative inflammation, *Lactobacillus* strains of the present preparation denature, decompose or assimilate as nutrients, and thus clean, exudates formed in the parts of the body in which symptons of infection, as it taught by pathology, are observed as a result of a battle against various kinds of foreign bodies, to cause the exudates to disappear, as will be apparent from what is stated above.

The present invention is characteristic in that, when administered orally, it can, in some cases, decrease the peculiar odor of excrement at the time of their evacuation. That is, even in an intestine containing more than 10^{11} cells/g of microbes forming their own peculiar mass of spores, the present preparation can proliferate well and predominate over said microbes. This is because the *Lactobacillus* strains of the present invention can grow faster than almost all intestinal bacteria, require low nutrients, can simultaneously produce antibiotics: and therefore can survive in growth, competition with intestinal bacteria. The odorous material in excrement comprise many types of compounds such as various amines, lower fatty acids, ammonia and sulfur compounds. When the amounts of such compounds exceeds a certain limit, they become poisonous to living bodies. The deodorizing effect of the present preparation for these compounds clearly indicates that it can digest or denature these materials thereby decreasing the amount of the latter or converting them to other materials. Moreover, these effects of the present preparation are obtained in the presence of a large amount of intestinal bacteria which produce the odorous materials, while

preventing the growth of such bacteria. Accordingly, said effect is one of the most important points of the present invention, and said characteristic effect or ability is displayed quite satisfactorily even in other affected parts or infectious diseases. Further, since the *Lactobacillus* strains of the present invention (which utilize as their nutrients the cells or exudate excreted by various part of the body) can grow very rapidly, the substitution thereof for other bacteria can proceed without hindrance from the latter.

Some of the examples of the medicinal treatment mentioned above indicate that the *Lactobacillus* strains of the present preparation can predominate over the pathogenic bacteria in the growth competition thereof with said bacteria. Further, the purification action of the present preparation has already been proven through the experiments of deodorization (Deodorization is one indication of the purification action). Thus, the *Lactobacillus* strains can temporarily proliferate profusely, but their growth may be minimized as the nutrient sources thereof (i.e., the exudate from the inflammed parts) disappear.

In summing up the effects of the present preparation: unlike the antibiotics which serve only to kill the bacteria, the present preparation is characteristic in that (a) it is non-pathogenic; (b) it can produce antibiotics thereby killing bacteria; (c) it can survive in the growth competition with pathogenic or other bacteria; (d) it denatures the metabolites (including poisonous ones) of living bodies or converts them into constituents of its own cells; (e) it purifies an affected part; (f) it shows anti-inflammatory or anti-swelling activities; and finally (g), as said preparation is foreign to the living bodies and is non-pathogenic, it is finally digested after the inflammatory diseases disappear. Accordingly, as is clear from the aforementioned discussions about the causes of the diseases or from the experimental data thereof, the present preparation can be used extensively to give better therapeutic effects, except where it is physically not possible to use it.

Moreover, we have ascertained that these therapeutic effects of the present preparation are sometimes increased when used in combination with enzymes having anti-inflammatory, anti-swelling or abating activities. Namely, although it is impossible to sweep away microbes by the use of the enzymes having such activities, and although antibiotics only alleviate the swelling induced by inflammatory diseases or have an insufficient effect thereon, diseases can be frequently improved very significantly by the use of the present preparation because of the bacterial substitution which takes place.

Turning now to the non-pathogenic property of the strains used in the present preparation. Originally, the history of bacteriology began with Pasteur's study of lactic acid bacteria. Despite that fact that many investigations about *Lactobacillus* have been carried out since 1857, it can be said that any scientific reports have not proven positively that this group of strains is pathogenic. Moreover, not only the current reliable dissertations show the *Lactobacillus* to be non-pathogenic, but Bergey's Manual (1974) also discloses specifically that pathogenic bacteria belonging to said groups are extremely rare.

According to the inventor's investigations, it has been proven that the presence of *Lactobacillus* is almost essential to the body, e.g., to the mucous membrane, particularly in the oral cavity, the intestine and the vagina. For example, it is almost impossible for normal conditions to be maintained in the vagina if *Lactobacillus* is not present therein. Therefore, whether or not *Lactobacillus* is present has recently been an important check-point in making a diagnosis of the health condition of the vagina. The present preparation shows a distinct effect upon deodorization of the vagina.

Although the strains which are used in the present invention belong to the group of *Lactobacillus*, whether or not they are pathogenic has not previously been known because they have previously unknown properties.

The fundamental problem to be determined first is whether or not the strains of the present preparation are in fact *Lactobacillus* strains. If they are *Lactobacilli* this fact alone indicates with a very high probability that they are non-pathogenic.

All the morphological properties of the new strains are, except for the nutritional requirements, identical with those of the known strains of *Lactobacillus*. The *Lactobacillus* strains known heretofore can be defined as gram-positive, facultative anaerobes and non spore-forming rods. Their shapes vary, depending on the strain, from spherical rod-like to curved rod-like, coryne-like or thread-like, but they do not form many branches. They are usually non-motile, negative to catalase, do not reduce nitrates, do not decompose gelatin and do not form indole or H_2S. Some strains are bipolar-stained. The ability of the *Lactobacillus* strains to decompose proteins and lipase is very poor, if not non-existent. They show better growth under an aerobic or slightly aerobic conditions than under fully aerobic conditions. They decompose sugar strongly and are acid-fast bacteria. Lactic acid is produced in a yield of more than 50% by the glucose fermentation thereof. According to the known morphological classification, the strains of the present preparation having these properties should be considered as belonging to the group of *Lactobacillus*. Moreover, the

morphology of microorganisms has not provided a clue to classify microbes according to the difference in the nutritional requirements thereof. At least at present, therefore, it is considered that the strains of the present preparation are *Lactobacillus* strains. The fact that the strains of the present preparation are considered to be *Lactobacillus* strains is of special importance in discussing the non-pathogenic property thereof.

The reason why we selected *Lactobacillus* in seeking deodorizing microbes for oral administration, despite the fact that other bacteria, such as strains of *Pseudomonas*, were thought to be available more easily, was that it was thought this group of microbes could provide useful strains, particularly as *Lactobacilli* are important members of intestinal bacteria. In fact, although at the initial stage of experiments, a great deal of concern was expressed that the deodorizing microorganisms isolated might, when administered orally, exert a bad influence on regular evacuation and other daily life, it was because of this concern about the non-pathogenic property and usefulness thereof that caused the continuation of the various experiments.

First of all, we carried out experiments using various dogs. Then, at the final state of the experiments, washed culture clots (wet 0.1 g/kg) were administered to both human subjects and dogs almost every day or sometimes at intervals of 2 to 3 days for 2 years. But no pathogenic effects were observed during the experiments, and the human subjects tested showed a decreased fatigue and an improvement in their health as subjective symptoms. Moreover, 2 dogs which had always been under the veterinary care recovered their health and were able to continue life without veterinary care.

Accordingly, the strains of the present preparation when administered orally to human beings show neither acute, sub-acute nor chronic toxicity. Moreover, when one mg/g of said strain suspended in 3-fold volume of a physiological saline solution was injected intraperitoneally to 50 mice, as compared with a control group the tested mice did not show any irregular symptons 24, 48 or 72 hours, one week, one month or 3 months after the administration. This indicates that the strains of the present preparation may not show any acute or sub-acute toxicity even by peritoneally administering 60 g of the clots of the microbial cells to human beings of 60 kg body weight. Therefore, the present preparation is considered to be substantially free from pathogenic property.

Next, the properties of the *Lactobacillus* strains of the present preparation isolated and cultivated herein, and the method of preparation thereof, are given hereinafter.

(a) *Bile resistance*

In order for the *Lactobacillus* strains to show their activity sufficiently in the intestines, it is preferable that said strains can grow in the presence of bile.

The bile resistance of the typical strain 1946 F.R.I. used in the present preparation is shown in Table I.

Other typical strains of the *Lactobacillus*, i.e., 2779/F.R.I., 2780/F.R.I., 2781/F.R.I. and 2782.F.R.I., which have been isolated successfully by the inventor and are usable in the present preparation show almost the same bile resistant properties as 1946/F.R.I. That is, they can proliferate well in a medium containing 4 by weight % of bile extracts. Of course, bile resistance is not essential because the present preparation may be used on parts in which bile is not present.

TABLE 1

Medium	Bile extracts 0 %	1 %	2 %	3 %	4 %
S-W medium + casamino acids	+	+−	+−	++	+
S-W medium + Na_2S	+	+−	+−	++	+
Meat extract bouillon	+	+−	+−	−−	+

Note: +, ++ and +− show the degree of growth
+ : good growth
++ : further good growth
+− : intermediate growth of + and ++
Components of S-W medium: KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.7 g, NaCl 1 g, $(NH_4)_2HPO_4$ 4 g, $FeSO_4 \cdot 7H_2O$ 0.03 g and glucose 5 g.
S-W medium indicates Stephenson-Whetham medium.

(b) *Nutritional requirements:*

Unlike the known strains of *Lactobacillus* which require amino acids, peptides, nucleic acids, vitamins, salts, fatty acids or their esters and sugars for their growth, the *Lactobacillus* strains of the present preparation show low nutritional requirements. Nevertheless, they show good growth within a short period of time (such as 2 days) and form lactic acid. Table 2 shows the degree of multiplication thereof in each of various media. S-W medium and S-W + Agar medium were used as the basic media therein.

TABLE 2

Compounds added to the basic medium	Basic medium	Strains (F.R.I. Nos.) 1946	2779	2780	2781	2782
No addition	(A)	−	−	−	−	−
	(B)	−	−	−	−	−
Sulfur-containing amino acids	(A)	+	−	−	+	−
	(B)	++	−	−	+	−
Cyclic amino acids	(A)	−	−	−	−	−
	(B)	−	−	−	−	−
Branched amino acids	(A)	−	−	−	−	−
	(B)	−	−	−	−	−
Cysteine	(A)	+	−	−	−	−
	(B)	++	−	−	+	−
Cystine	(A)	+	−	−	−	−
	(B)	++	−	−	+	−
Methionine	(A)	+	−	−	−	−
	(B)	+	−	−	+	−
Casamino acids	(A)	+	−	−	+	−
	(B)	++	+	+	+	+
Casamino acids + Vitamins	(A)	+	+	+	+	+
	(B)	++	+	+	++	+
Casamino acids + yeast extracts	(A)	+−	+	+	+	+
	(B)	++	+	+	++	+
Yeast extracts	(A)	+	+	+	+	+
	(B)	++	+	+	++	+

Note: (A) : S-W medium
(B) : S-W medium (+ Agar)
\+ : Normal growth
++ : Good growth
+++ Very good growth
− : Poor growth
− : No growth

(c) *Specific growth rate:*

As seen in Table 3, the *Lactobacillus* strains of the present preparation show surprisingly high specific growth rates even in media of low nutritious conditions. Just for comparison, the specific growth rate of *Escherichia coli* are shown in the table.

TABLE 3

Nutritional requirements and relative growth rate Basic medium: S-W medium)

Strain Nos. F.R.I.	Ingredients added to the basic medium amino acids	S,N,C and sulfur-containing	μ	μ in case of *Escherichia coli*
1946	Sulfur-containing amino acids	Yes	0.53	0.4
2779	Vitamines, sulfur-containing amino acids	Yes	0.46	0.43
2780	Vitamins	Yes	0.46	0.38
2781	S,N,C	Yes	0.53	0.35
2782	Vitamins, sulfur-containing amino acids	Yes	0.46	0.43

Note
S : S-compounds — Na_2S or H_2S
N : N-compounds — ammonia, indole or scatole
C : C-compounds — lower fatty acids e.g.(acetic acid or butyric acid)

Yes: essential for growth

Thus, although the known strains of *Lactobacillus* show high nutritional requirements and lower growth rate as compared with pathogenic bacteria, the *Lactobacillus* strains of the present preparation can predominate over the generally known pathogenic bacteria in the growth competition therewith.

(d) The results of microscopic observation and the morphological characteristics of the *Lactobacillus* strains of the present preparation are shown in Table 4. Tables 5 and 6 show the biochemical properties and the ability to decompose sugars, respectively.

TABLE 4

Microscopic observation and morphological characteristics

	F.R.I. Nos 2779	2780	2781	2782	1946
Gram	+	+	+	+	+
Shape	short rod ,rounded ends, no flagella and no spore	cocco-bacilli, no flage-lla and no spore	cocco-bacilli, no flage-lla and no spore	short rod ,rounded ends, no flagella and no spore	short rod ,rounded ends, no flagella and no spore
Capsule	No	No	No	No	No
Motility	No	No	No	No	No
O_2	anaero-bic	anaero-bic	anaero-bic	anaero-bic	anaero-bic
In a medium of normal broth agar medium + sugar + vitamins	round middle colonies	round middle colonies	round middle colonies	round middle colonies	round middle colonies
Projection	Semi-spherical	Semi-spherical	Thin	Thick	Thick
Surface	smooth moistened	smooth moistened	smooth moistened	smooth moistened	smooth moistened
Circum-ference	Plain	plain	plain	plain	plain
Color	milky white, not transpa-rent. mucous	milky white, not transpa-rent. mucous	white. not transpa-rent. mucous	milky white, not transpa-rent, mucous	milky white, not transpa-rent. mucous

TABLE 5

(General properties)

	F.R.I. Nos				
	2779	2780	2781	2782	1946
Ammonia-production	–	–	–	–	–
H_2S-production	–	–	–	–	–
Catalase-production	–	–	–	–	–
Pigment-production	–	–	–	–	–
Gelatin liquefaction	–	–	–	–	–
Utilization of citric acid	–	–	–	–	–
Decomposition of urea	–	–	–	–	–
M.R. reaction	+	+	+	+	+
V.P. reaction	–	–	–	–	–
Reduction of nitrates	–	–	–	–	–

TABLE 6

(Ability to decompose sugars)

	F.R.I. Nos.				
	2779	2780	2781	2782	1946
Glucose	+	+	+	+	+
Galactose	+	+	+	+	+
Fructose	+	+	+	+	–
Salicin	+	+	+	+	–
Arabinose	–	+	–	–	–
Xylose	–	–	–	+	–
Sucrose	+	+	+	+	+
Inositol	–	–	–	–	–
Dextrin	+	+	±	±	–
Mannitol	–	–	–	–	–
Melebiose	+	+	+	+	+
Ribose	+	+	+	+	–
Lactose	+	+	+	+	+
Raffinose	–	–	+	+	–
Starch	+	+	+	+	+
Inuline	–	–	–	–	–
Sorbitol	–	–	–	+	–
Maltose	+	+	+	+	+
Melezitose	–	–	–	–	–
Mannose	+	±	+	+	–

Antibiotic-production

Although some strains of *Lactobacillus* have been known to show an antibiotic production. all the strains specified herein have this antibiotic production. This antibiotic production of the preferred *Lactobacillus* strains serves to prevent the growth of other bacteria or the formation of pus. sputum. serum and other poisonous material.

Table 7 shows one example of the inhibitory effects against bacteria which were estimated by placing a trace of *Lactobacillus* strain of the present preparation at the center of a petri dish containing (normal broth agar medium + sugar + vitamins)-medium. cultivating it at 37°C for 2 days. and then spreading *Staphylococcus aureus* (as a representative example of gram-positive bacteria) or *Escherichia coli* (as a representative

example of gram-negative bacteria) on the medium. In actual cases, however, depending on the components of the media and the methods of cultivation or storage, it may sometimes happen that the strains of the present invention show greater antibiotic production than those indicated in Table 7 or do not show any antibiotic production.

TABLE 7

Strains (F.R.I. Nos.)	Bactericidal activity Inhibition diameter (pre-cultivated for 48 hours	
	Staphylococcus aureus	Escherichia coli
1946	20 mm	24 mm
2772	25 mm	18 mm
2780	15 mm	20 mm
2781	12 mm	15 mm
2882	18 mm	22 mm

(f) Table 8 shows that the *Lactobacillus* strains used in the present preparation are, though varying with the basic media employed, promoted in their growth by the addition various odoriferous ingredients of excrement to the media. Additionally, similar results were obtained by adding S, N or C-containing substances other than those shown in said Table.

The fundamental bacteriological differences between known *Lactobacillus*, strains and those of the present preparation will now be explained using the data shown in Table 9-(a), Table 9-(b) and Table 9-(c). First of all, the degree of multiplication of known *Lactobacillus* strains and those of the present preparation under low, middle or high nutritional conditions as well as the changes or degree of changes of their multiplication in the presence of acetic acid are shown in Table 9-(a). This table indicates the clear difference between both groups. Thus, although the addition of a suitable amount of acetic acid to a good nutritional medium (e.g., Briggs' medium which is a typical one for *Lactobacillus)* is known to promote the growth of the known strains of *Lactobacillus*, such phenomenon can be observed only in good nutritional media. In other words, since known *Lactobacillus* strains cannot grow in a low nutritional medium, the addition of acetic acid never serves to stimulate the growth thereof. On the contrary, multiplication of the *Lactobacillus* strains of the present preparation is strongly promoted by adding a suitable amount of acetic acid to the low or relatively low nutritional media shown in Table 9-(a); but in good or relatively good nutritional media the degree of stimulation to the growth is slight or even non-existent by addition of acetic acid.

Moreover, as seen in Table 9-(b), when known *Lactobacillus* strains and those of the present preparation are cultivated in low, middle or high nutritional media containing $Na_2S \cdot 9\,H_2O$ or NH_3, the multiplication of the strains of the present invention in low middle nutritional media is stimulated by addition of 0.1 g or 1 g of $Na_2S \cdot 9H_2O$, whereas said addition to the low, middle or high nutritional media does not stimulate the growth of known *Lactobacillus* strains.

Further, the multiplication of the *Lactobacillus* strains of the present preparation in low or middle nutritional media is stimulated by the addition of NH_3, whereas the growth of known *Lactobacillus* strains is minimized by addition of even a small amount (e.g. 1 g/l) of $Na_2S \cdot 9H_2O$ or ammonia.

Thus, it is clear that, unlike the known strains, the *Lactobacillus* strains of the present preparation show new and special behaviors to Na_2S and ammonia under low or medium nutritional conditions.

Furthermore, the multiplication of the *Lactobacillus* strains of the present preparation in low, middle or high nutritional media are stimulated by addition of a mixture of $Na_2S \cdot 9H_2O$, ammonia and acetic acid, whereas the addition of said mixture to low, middle or high nutritional mediua never serves to stimulate the multiplication known strains of *Lactobacillus*(Table 9-(c)).

Also in Tables 9-(a) to (c) it should be noted that the *Lactobacillus* strains of the present preparation, explained above as those whose growth is promoted in the presence of S.N. and C-containing substances, include a group of strains which can only grow in the presence of said substances.

TABLE 8

Compounds added to the basic medium	Basic medium	Strains (F.R.I.Nos) 1946	2779	2780	2781	2782
No addition	A	−	−	−	−	−
	B	−	−	−	−	−
	C	+	+	+	+	+
	D	++	++	++	++	++
Acetic acid	A	−	−	−	−	−
	B	+	−	−	+	−
	C	+−	+−	+−	++	+−
	D	++	++	++	++	++
Ammonia	A	−	−	−	−	−
	B	+	−	−	−	−
	C	+−	+−	+−	++	+
	D	++	++	++	++	++
Propionic acid	A	−	−	−	−	−
	B	+	−	−	+	−
	C	+−	++	+−	++	+−
	D	++	++	++	++	++
$Na_2S \cdot 9H_2O$	A	−	−	−	+	−
	B	++	−	−	+	−
	C	++	+−	+−	++	++
	D	++	++	++	++	++
Butyric acid	A	−	−	−	−	−
	B	+	−	−	+	−
	C	++	++	+−	++	+−
	D	++	++	++	++	++
Scatole	A	−	−	−	−	−
	B	+	−	−	−	−
	C	+	+	+−	+−	+
	D	++	++	++	++	++
Excremental juice	A	+	+	+	+	+
	B	+−	+	+	+	+
	C	++	+−	+−	+−	+−
	D	++	++	++	++	++

Note: (A): S-W medium
(B): S-W medium (+ Agar)
(C): Peptone 8 g + Glucose 2 g
(D): Peptone 10 g + Meat extract 5 g + NaCl 5 g + Glucose 1 g.

TABLE 9-(a)

Basic medium		Degree of stimulation of *Lactobacillus* Known strains	Strains of the invention	Amount of acetic acid added (g/liter)	Degree of stimulation of *Lactobacillus* Known strains	Strains of the invention
Low nutrition		−	+	1	−	+
				2	−	+−
				5	−	++
Middle nutrition	Low	−	+	1	−	+
				2	−	+
				5	+	+−
	Middle	−	+	1	−	−
				2	−	−
				5	+	+
	High	−	++	1	−	−
				2	+	−
				5	+−	+
High nutrition		+	++	1	−	−
				2	−	−
				5	−	−

TABLE 9-(b)

Basic medium		Amount of $Na_2S \cdot 9H_2O$ added (g/l)	Degree of stimulation of *Lactobacillus* in the presence of $Na_2S \cdot 9H_2O$ Known strains	Strains of invention	Amount of ammonia added (g/l)	Degree of stimulation of *Lactobacillus* in the presence of ammonia Known strains	Strains of invention
Low nutrition		0.1	−	−	0.1	−	−
		1	−	−	1	−	−
		2	−	−	2	−	−
Middle nutrition	Low	0.1	−	−	0.1	−	−
		1	−	−	1	−	−
		2	−	−	2	−	−
	Middle	0.1	−*	−	0.1	−	−
		1	−*	+	1	−*	−
		2	−*	−*	2*	−*	−
	High	0.1	−	−	0.1	−	−
		1	−*	+	1	−*	−
		2	−*	−*	2	−*	−
High nutrition		0.1	−	−	0.1	−	−
		1	−*	−	1	−	−
		2	−*	−*	2	−*	−

Note: −*: bacterial growth suppression.

TABLE 9-(c)

Basic medium	Amount of compounds added (g/l) $Na_2S \cdot 9H_2O$	NH_3 Acetic acid	Degree of stimulation of *Lactobacillus* in the presence of S, N, C substances	Known strains	Strains of the invention
Low nutrition	0.1	+ 0.1	+ 0.1	−	−
	1	+ 1	+ 1	−	+
	2	+ 2	+ 2	−	−*
Middle nutrition — Low	0.1	+ 0.1	+ 0.1	−	−
	1	+ 1	+ 1	−*	+
	2	+ 2	+ 2	−*	−*
Middle nutrition — Middle	0.1	+ 0.1	+ 0.1	−	−
	1	+ 1	+ 1	−*	−
	2	+ 2	+ 2	−*	−*
Middle nutrition — High	0.1	+ 0.1	+ 0.1	−	−
	1	+ 1	+ 1	−*	−
	2	+ 2	+ 2	−*	−*
High nutrition	0.1	+ 0.1	+ 0.1	−	−
	1	+ 1	+ 1	−*	−
	2	+ 2	+ 2	−*	−*

The low, middle and high nutritional media shown inTable 9 are each defined as those which are obtained by classifying the nutritional requirements of known *Lactobacillus* strains or those of the present preparation into three groups, and the middle nutrition into further three groups, while taking into account the biological properties thereof. More specifically, the low nutritional medium shown herein refers to a medium containing (S-W) + vitamins or (S-W) +Casamino acids (vitamin-free) at most. Of course, in the medium, other specific vitamins or amino acids may be used in place of said vitamins or casamino acids; or alternatively a medium not containing both of said ingredients may be used. Namely, the low nutritional medium described herein should be interpreted as referring to all the media which do not contain more nutrients than those described above.

On the other hand, the low nutrition of the middle nutritional medium shown in Table 9 refers to (S-W) + vitamins + sulfur-containing amino acids; and the middle nutrition of the middle nutritional medium refers to (S-W) + vitamins + casamino acids; peptone + sugars, or a medium of almost the same nutritional value. The high nutrition of the middle nutritional medium refers to those which consist of the same ingredients as those of the high nutritional medium but which contain only 1/5 to 1/3 nutriments of the latter. In this connection, however, a medium in which some other vitamins and amino acids are added to (S-W)-medium in place of the vitamins and casamino acids used may also be employed as the middle nutritional medium.

Further, the high nutritional medium stated hereinbefore refers to any of the media which are, as already disclosed in various scientific reports, especially suitable for proliferation of the known *Lactobacillus* strains. And such media include not only MRS-medium, but also those which contain amino acids, peptides, nucleic acids, vitamins, minerals, fatty acids or their esters and/or sugars in good nutritional proportions suitable for growth of known *Lactobacillus* strains.

Anyway, it should be understood that the *Lactobacillus* strains of the present preparation are not limited to the five strains mentioned above but include any strains which have the same morphological characteristics and nutritional requirements as defined hereinbefore, though the therapeutic effects thereof may vary depending on the actual strain. Additionally, while the antibiotic-productivity of the strains of the present preparation is important in aiding the therapeutic effects thereof indirectly, it has been ascertained in our experiments that strains having no such productivity also show their effects quite satisfactorily.

The following Examples illustrate making the present preparation.

(i) A *Lactobacillus* strain having the hereinbefore defined morphology and nutrional

properties was inoculated into a medium (pH 7.4) containing the following ingredients:

Skim milk
Yeast extract
$CaCO_3$

The medium was cultivated by allowing it to stand at 37°C for 3 days. Then, the medium was centrifuged under cooling and the collected microbial cells were freeze dried in vacuo, whereby a pharmaceutical *Lactobacillus* preparation was obtained.

(ii) A *Lactobacillus* strain having the hereinbefore defined morphology and nutritional properties was inoculated into a medium (pH 7.4) containing the following ingredients:

S-W medium*

$Na_2S \cdot 9H_2O$

Components of S-W medium:
1g KH_2PO_4; 0.7g $MgSO_4 \cdot 7H_2O$; 1g NaCl; 4g $(NH_4)_2HPO_4$; 0.03g $FeSO_4 \cdot 7H_2O$; and 5 g glucose.

*S-W medium indicates Stephenson-Whetham medium.

Propionic acid
Butyric acid
Yeast extracts
Vitamins
Amino acids

The medium was cultivated by allowing it to stand at 37°C for 3 days. Then, the microbial cells were carefully dried until the water content thereof becomes 2%.

The following Examples illustrate the use of the present preparation,

Example 1

The present preparation was administered to three patients suffering from acture sinusitis, five patients suffering from chronic sinusitis and two patients suffering from post-operative sinusitis by dissolving 20 g of the above preparation (water content: 2%) in 400 ml of water, and washing the nasal sinuses of the patients with the resultant solution twice a day for 21 consecutive days. Further, for two patients who had a viscous nasal mucus discharge, the present preparation was used together with tetracycline. Based on (1) subjective symptons (rhinostenosis, post-nasal discharge, nasal discharge, depression in the sense of smell and headache), (2) a pathological observation of nasal sinus and nasal mucous membranes (colour of mucous membranes swelling, amount of nasal discharge, nature of nasal fluids) and (3) direct and X-ray examination, the therapeutic effects of the present preparation were estimated as 4 points (remarkably effective) 2 points (effective), 1 point (slightly effective) and 0 point (ineffective). Table 10 shows the effects of the present preparation at one and 3 weeks after the treatment was started.

TABLE 10

(One week after the treatment was started)

No.	Initial of Name	Age	Sex	Name of disease	Additional Drug used	Subjective symptom	Pathological observation	X-ray	Average
1	M.O.	10	♂	Acute sinusitis	none	2	1	1	1.3
2	T.M.	25	♀	"	"	2	2	1	1.7
3	K.H.	44	♂	"	"	1	1	1	1
4	K.A.	20	♂	Chronic sinusitis	"	2	2	2	2
5	N.N.	36	♀	"	anti-biotic	2	1	1	1.3
6	Y.E.	62	♂	"	none	1	1	1	1
7	K.Y.	51	♀	"	"	1	1	1	1
8	Y.M.	18	♀	"	"	2	2	2	2
9	T.T.	26	♂	Post-operative sinusitis	antibiotic	1	2	1	1.3
10	S.O.	42	♂	"	none	2	1	2	1.7

(Three weeks after the treatment was started)

No.		Subjective symptom	Pathological observation	X-ray	Average
1		4	2	2	2.7
2		4	4	2	3.3
3		2	2	2	2
5	Same as above	2	2	1	1.7
6		2	2	1	1.7
7		2	2	2	2
8		2	2	2	2
9		2	4	2	2.7
10		4	2	2	2.7

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As seen in the tables, when the present preparation was used for the ten patients, said preparation proved to be remarkably effective for the treatment of the sinusitis in one of the patients; effective in six patients; and slightly effective in two patients. Further, in this example, no case was observed in which the present preparation was ineffective in all of the three items of the examination.

Example 2

Patients treated: 10 patients suffering from hemorrhoids who were mainly afflicted with pain, swelling or bleeding (16 - 60 years old).

Methods of Application: (A)....the dried cells water content: 2%) of a *Lactobacillus* strain having the necessary morphology and nutritional properties and additionally which was resistant to tetracycline. The preparation was administered orally 5 times a day (Dose: 3 g per each time).

(B)....The same preparation was mixed with half its volume of an ointment and applied to the affected part 5 times a day.

(C)....An ointment containing tetracycline was applied to an affected part prior to application of the present preparation.

The tests were first carried out by the following three methods; i.e., (C) + (A), (C) + (B) and (C) + (B) + (A).

Judgement as to their effects: Based on the subjective symptoms and the secondary observations such as subjective pain, bleeding, swelling, the degree of hemorrhoidal nodule and a sense of incongruity at the anus, the therapeutic effects was ranked as +4 (remarkably effective), +2 (effective), +1 (slightly effective), 0 (ineffective) and-2 (aggravated).

The therapeutic effects were observed in 7th, 14th, and 21th day. Further, Table 11 shows the results which were carried out according to (A) + (B) + (C) and were estimated in 7th and 21th day after the treatment was started.

TABLE 11

(One week after the treatment was started)

Name	Subjective pain	Bleeding	Swelling	Degree of hemorrhoidal nodule	Feeling of Discomfort	Average
Y.T.	1	1	1	1	1	1
K.H.	2	2	1	1	1	1.4
M.M.	4	4	2	2	2	2.8
T.S.	1	1	1	1	1	1
T.H.	1	1	1	1	1	1
K.M.	2	1	1	1	1	1.2
M.O.	2	2	2	2	1	1.8
S.S.	1	1	1	1	1	1
N.H.	1	2	1	2	1	1.4
T.Y.	1	1	1	1	1	1

The results were characteristic in that there was not observed any cases of "ineffective" and "aggravated".

(Three weeks after the treatment was started)

Name	Subjective pain	Bleeding	Swelling	Degree of hemorrhoidal nodule	Feeling of Discomfort	Average
Y.T.	2	2	2	2	2	2
K.H.	2	2	2	2	2	2
M.M.	4	4	4	2	4	3.6
T.S.	1	1	1	1	1	1
T.H.	2	2	2	2	2	2
K.N.	2	2	2	2	1	1.8
M.O.	4	4	2	2	2	2.8
S.S.	2	1	1	1	1	1.2
N.H.	2	2	2	2	2	2
T.Y.	2	1	1	1	1	1.2

The characteristics of this test results was that there was no case which showed "4" or "0" in all of the five examination items.

Example 3

Table 12 shows the result of the clinical tests which were carried out in the field of the dentistry by the use of the present preparation. The tests were carried out by

(i) packing it (2% dried cells) directly into the affected part,

(ii) suspending the present preparation in a physiological saline solution, and then injecting said solution with a syringe,

(iii) gargling the throat with an aqueous suspension of the present preparation, or

(iv) applying an ointment containing the present preparation to the effected part.

The results were indicated as +++ (remarkably effective), ++ (fairly effective), + (effective) and - (ineffective).

TABLE 12

No.	Name of patient	Sex	Age	Position and symptom	Method of operation	Method of administration	Effects
1	K.S.	♂	37	1) gingival abscess	Extraction of tooth	(i)	++
2	T.A.	♂	25	1) "	none	(ii)	+
3	H.O.	♀	46	6) alveolar abscess	none	(iv)	+++
4	E.M.	♀	22	8) "	none	(iv)	++
5	S.I.	♂	19	8/8 periodon titis	Extraction of tooth	(i)	++
6	T.H.	♀	40	7) "	"	(ii)	+
7	T.M.	♂	57	8) wisdom tooth inflamm-ation	"	(iii)	++
8	S.N.	♂	60	8/8 "	none	(iv)	+++
9	M.N.	♂	21	6) gingival abscess	none	(iii)	++
10	S.T.	♂	34	1) "	none	(ii)	++
11	M.K.	♀	30	7) "	Extraction of tooth	(i)	+
12	M.M.	♀	27	8) pulpitis	"	(i)	+

As is clear from the table. the high therapeutic effects were observed in almost all of the patients.

Example 4

The present preparation was used for the treatment of the pudendal laceration and the swelling or pain shown after pudendal operations. That is, in the tests on ointment containing the preparation was applied to the affected part several times a day. Further, in the case of heavy laceration, the present preparation was used together with antibiotics and protease. The results are shown in Table 13.

TABLE 13

No.	Name	Age	Child birth	Symptoms	Degree of swelling	Degree of pain	Sewing up	A* (g/day)	T** (times	Observation
1	W.M.	26	first	pudendal laceration	++	++	pudendal vagina	3	5	2 days later: swelling and pain alleviated; good sewing up
2	K.K.	24	"	"	+	"	"	"	"	
3	M.A.	30	"	"	++	"	"	"	4	2 days later; swelling alleviated. 4 days later: pain alleviated; good sewing up
4	J.S.	21	"	"	++	"	"	"	5	"
5	M.M.	29	"	incision of pudenda	++	"	"	3 (antibiotic and protease)	4	"

Note: A* : Amount applied (g/day)
T**: Number of times applied (times/day)
The treatment was effective in all of the five patients.
They convalesced satisfactorily.

Despite the discovery of strong antibiotics, the above-mentioned diseases still belong to a group of diseases which are recognized as being difficult to cure. Accordingly, it is inferred that the present preparation is equally applicable to other infectious diseases which are induced by substantially the same mechanisms as the above-mentioned ones.

Example 5

After appendectomy, the present preparation was used for the removal of pathogenic bacteria, fibrin produced at the local section, dead tissues, pus and so forth, or for the treatment of pathogenic bacteria-induced gastritis and enteritis. Said preparation was administered orally and, in almost all cases, together with antibiotics. In comparison untreated patients, the results were indicated as +++ (remarkably effective), ++ (fairly effective), + (effective) and - (ineffective). The *Lactobacillus* strain used in their experiments was one which is also resistant to the antibiotics used, and in the case of adult patients, the present preparation (the fresh cultivation broth) was administered 8 times a day at a dosage each time of 3 ml/kg of body weight.

TABLE 14

No.	Sex	Age	Name of operation	Remarks	Conditions after the operation	Effects
1	♂	38	removal of appendix		good	++
2	♂	57	"		"	+
3	♀	40	"		"	+
4	♀	29	"	"	++	
5	♂	35	enteritis	*Vibrio*	"	++
6	♀	32	"	"	"	+
7	♂	18	"	*Salmonella*	"	+
8	♂	24	"	"	"	+++

Table 14 clearly shows that all the patients convalesced satisfactorily and the present preparation had a good therapeutic effect. Although it sometimes happens in appendicitis cases that the wound could not be sewn up well or the surgical operation had to be repeated because of distribution of bacteria around the affected part or the insufficient inhibitory effects of antibiotics used against bacteria, such incidents were not observed during the experiments shown in the tables or during other various experiments connected therewith.

From the foregoing, it will be manifest that the *Lactobacillus* strain(s) used in the preparation according to the present invention can be isolated from other *Lactobacilli* by a technique which includes subjecting the *Lactobacilli* mixture to nutrient conditions under which strains other than the required *Lactobacilli* strains do not grow but under which the required stains do grow.

In this Specification, the F.R.I. Nos. 1946, 2779, 2780, 2781 and 2782 refer to the deposit numbers of the microorganisms at the Fermentation Research Institute where they are referred to officially as Form P Nos. 1946, 2779, 2780, 2781 and 2782, respectively.

Attention is drawn to the specification and claims of our copending British Patent Application No. 21344/77 which relates to compositions useful for culturing and storing a *Lactobacillus* strain and to a deodorizing composition containing living cells of a *Lactobacillus* strain.

WHAT WE CLAIM IS:-

1. A pharmaceutical *Lactobacillus* preparation useful for the prevention of infection or inflammation or combatting of inflammation of infectious disease comprising one or more strains of live *Lactobacillus* whose growth is enabled or promoted by addition of one or more of sodium sulphide, ammonia and acetic acid to at least one of Stephenson-Whetham medium, Stephenson-Whetham medium containing vitamins and Stephenson-Whetham medium containing casamino acid, said preparation being substantially free of other bacterial strains.

2. A pharmaceutical *Lactobacillus* useful for the prevention of infection or inflammation or combatting of inflammation or infectious disease preparation comprising one or more strains of live *Lactobacillus* whose growth is enabled or promoted by addition of one or more of sodium sulphide, ammonia and acetic acid to at least one of Stephenson-Whetham medium, Stephenson-Whetham medium containing vitamins and Stephenson-Whetham medium containing casamino acid; and a carrier and/or excipient.

3. A preparation as claimed in Claim 1 or 2, wherein said strains of *Lactobacillus* can grow in the presence of bile.

4. A preparation as claimed in any preceding claim, wherein said strains of

Lactobacillus show antibiotic production.

5. A preparation as claimed in any preceding claim, wherein said strains of *Lactobacillus* can grow in the presence of antibiotics.

6. A preparation as claimed in Claim 1 or 2, wherein said strains of *Lactobacillus* is/are one or more of the strains 1946/F.R.I., 2779/F.R.I., 2780/F.R.I., 2781/F.R.I., and 2782/F.R.I.

7. A method of treatment of a non-human mammal for the prevention of infection or inflammation or combatting of inflammation or infectious disease, comprising administering a preparation as claimed in any preceding claim to said non-human mammal.

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Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1981.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.